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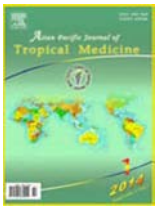

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Antioxidant capacity and total phenolic contents in hydrophilic extracts of selected Bangladeshi medicinal plants

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ABSTRACT

Objective: To evaluate hydrophilic extracts from edible portions of fifteen plants for total phenolic content (TPC) and anti-oxidant capacity (AC) as an effort to find possible sources for future novel antioxidants.

Methods: Folin-Ciocalteau and DPPH radical scavenging assays were employed to determine TPC and AC, respectively.

Results: Among the assayed plants, TPC (mean±SD), expressed as gallic acid equivalent, varied from 0.04±0.01 (*Amaranthus spinosus*) to 6.01±0.04 (*Zanthoxylum rhetsa*) mg gallic acid equivalent/g fresh weight. AC (mean±SD), expressed as trolox equivalent, ranged from 0.14±0.00 (*Alternanthera philoxeroides*) to 7.54±0.00 (*Zanthoxylum rhetsa*) μmol trolox equivalent/g fresh weight. A significant and positive linear relationship (R^2=0.99) was observed between TPC and AC of *Zanthoxylum rhetsa*, *Oxalis corymbosa*, and *Alternanthera sessilis*.

Conclusions: The results of the present study implies that the analyzed plants possess varying degree of antioxidant capacity and, therefore, the antioxidant potency of these underused plants may be utilized to prevent oxidative damage and oxidative stress related disorders.

1. Introduction

Non-communicable diseases (NCDs) are increasingly becoming the major threats to the health care systems in the world. Reactive nitrogen and oxygen species like nitric oxide, singlet oxygen, hydrogen peroxide, superoxide, hydroxyl and peroxyl free radicals are widely considered to be the critical part of this epidemiological transition. This is because of the capacity of these reactive species, when accumulated within body in excess amount, to cause oxidative stress, one of the prominent etiological factors leading to chronic inflammation, cancer, atherogenesis and vascular disease, neurological, pulmonary and age-related pathology and diseases[1-3]. Since there is hardly any cure for these health problems, a food-based preventive approach is generating considerable interest among the scientists in terms of its potentiality in tackling the degenerative or pathological processes leading to NCDs. As a preventive approach, scientists are looking for the use of bioactive compounds like polyphenolic compounds from various plant extracts.

In this context, medicinal plants attract special attention and, are being surveyed for their potential roles in different regions of Bangladesh[4]. The World Health Organization has also estimated conservatively that 60 to 90 percent of the populations of the non-industrialized countries rely, either totally or partially, on medicinal plants to meet their health care needs[5]. Scientists have reported that polyphenolic compounds significantly constitute to the active substances in these plants' extracts having multiple protective effects including antioxidant[6], anti-inflammatory[7], antibacterial[8] and antiproliferative activities[9]. These

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phenolic compounds have been proven to possess a wide range of biological activities both *in vitro* and *in vivo*. They have powerful antioxidant properties like prevention of free radicals induced oxidative damage as well as different anticarcinogenic, vasodilatory, hypoglycemic and neuroprotective effects[10-12]. Experimental and epidemiological studies supported that diet rich in phenolics (derived from both plant and plant products) are associated with lower risk of NCDs, particularly cancer, cardiovascular diseases and neurodegenerative diseases[13]. As a result, phenolic compounds have received tremendous attention among nutritionists, food scientists, pharmacists and among consumers as well.

Consequently, data on phenolic contents and antioxidant properties have been generated to a considerable extent in Bangladesh likewise around the globe. A recent review of the literature on this topic provided total phenol content (TPC) and antioxidant capacity (AC) related data on different varieties of fruits, rice, vegetables and indigenous foods[14-17]. However, no study regarding the polyphenols content and/or antioxidant properties of the local underused medicinal plants was found. So, the present research was undertaken to screen fifteen commonly consumed medicinal plants TPC and AC. Furthermore, the relationship between TPC and AC of these plants' extracts was also examined in this study.

2. Materials and methods

To screen the TPC and AC, 15 medicinal plant samples were collected from the medicinal garden of Department of Botany, University of Dhaka and local market. The study samples included slender amaranth [*Amaranthus viridis* (*A. viridis*)], sessile joyweed [*Alternanthera sessilis* (*A. sessilis*)], alligator weed [*Alternanthera philoxeroides* (*A. philoxeroides*)], spiny amaranth [*Amaranthus spinosus* (*A. spinosus*)], sickle senna [*Senna tora* (*S. tora*)], Indian penny wort [*Centella asiatica* (*C. asiatica*)], Bengal dayflower [*Commelina benghalensis* (*C. benghalensis*)], drumstick tree leaves [*Moringa oleifera* (*M. oleifera*)], curry leaves [*Murraya koenigii* (*M. koenigii*)], creeping wood-sorrel [*Oxalis corniculata* (*O. corniculata*)], pinkwood sorrel [*Oxalis corymbosa* (*O. corymbosa*)], gooseberry [*Physalis angulata* (*P. angulata*)], Javanese long pepper [*Piper retrofractum* (*P. retrofractum*)], para cress [*Spilanthes calva* (*S. calva*)], Indian Ivy-rue [*Zanthoxylum rhetsa* (*Z. rhetsa*)]. All the samples were collected fresh and processed for analysis in the Food Analysis Laboratory of Institute of Nutrition and Food Science, University of Dhaka, Bangladesh. The portion of the plants used for analysis is given in the Table 1.

Table 1
Edible parts of the plants used for analysis.

Name	Edible parts analyzed
A. viridis, *A. philoxeroides*, *A. sessilis*, *A. spinosus*, and *C. benghalensis*	Tender stem, shoots and leaves
S. tora, *M. koenigii*, *S. calva* and *M. oleifera*	Tender leaves
C. asiatica	Tender stem and leaves
O. corniculata and *O. corymbosa*	Whole plant except root
P. retrofractum	Stem
Z. rhetsa	Leaves
P. angulata	Tender stem, leaves & fruits

2.1. Sample preparation

The edible portion of the samples were separated immediately after collection, and washed under running water followed by distilled water. They were drained completely and air dried over filter paper. Then the samples were cut into small pieces (<0.25 cm) to increase the surface area which facilitated freeze drying. The samples were then weighed and freeze dried (il Shin lab. Co. Ltd., Korea). The freeze dried samples were weighed and homogenized using a grinder. The ground samples were stored in air-tight packets at -20 °C in a refrigerator prior to extraction.

2.2. Chemicals and reagents

n-Hexane (MERCK, Germany), dichloromethane (MERCK, Germany), acetone (MERCK, Germany) and acetic acid (MERCK, Germany) were used for solvent extraction. Gallic acid (TIC, Japan), sodium carbonate and Folin-Ciocalteau reagent (FCR) (MERCK, Germany) were used for estimation of total phenol. 2-(N-morpholino)ethanesulfonic acid buffer (DojinDo), 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) (Wako, Japan), Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, a vitamin E analogue) (ALDRICH, Denmark) and ethanol (MERCK, Germany) were used for estimation of antioxidant capacity. All chemicals used for the analysis were of analytical grade.

2.3. Instruments

A shaker (Controlled Environmental Incubator Shaker, New Brunswick Scientific Co INC; Edition-N.J.U.S.A.), a centrifuge machine (Hettich Universal II), and a sonication bath (ULTRASONS MEDI-II) were used for sample extraction. An UV-VIS spectrophotometer (UV-1800, Schimadzu, Japan) was used to record absorbance at specific wavelengths (520 nm and 750 nm).

2.4. Sample extraction

One gram freeze dried ground sample was taken and 25 mL hexane: dichloromethane (1:1) was added to it. Then it was allowed to shake overnight in a shaker at 100 r/min at room temperature. After completion of shaking, the mixture was centrifuged at 2 500 r/min for 15 min. The supernatant was discarded and the precipitate was dried at 55 °C to evaporate the left over solvent. To the precipitate, 25 mL of acetone: water: acetic acid (70:29.5:0.5) was added and then sonicated at 25 °C for 15 min by using sonication bath for disrupting the cell matrix of the samples to facilitate the maximum extraction of phenolic compounds from the samples. After sonication, the sample was again centrifuged at 2 500 r/min for 15 min. The supernatant was then poured to a 25 mL volumetric flask. Acetone: water: acetic acid was added to make the final volume to 25 mL. Subsequently, these extracts were then stored in a refrigerator at −20 °C until analysis.

2.5. Determination of TPC in plant extracts

TPC of the selected medicinal plants' extracts was estimated colorimetrically according to the Folin-Ciocalteau method[18]. For each sample, 100 μL of sample extracts were put into 3 drum vials. Then 600 μL distilled water was added to each vials. To these, 150 μL of two times diluted Folin-Ciocalteau reagent were added and allowed to stand for 5 min at ambient temperature. After 5 min, 750 μL of 2% sodium carbonate was added and allowed to react for 15 min at ambient temperature. The absorbance was estimated at 750 nm with UV-VIS spectrophotometer. A gallic acid standard curve of varying concentrations was constructed by plotting gallic acid concentration on abscissa and absorbance on ordinates for quantification of total phenol. The TPC was expressed as gallic acid equivalent per gram of fresh weight (mg GAE/g FW).

2.6. Estimation of AC of plant extracts

Estimation of the AC was performed by DPPH radical scavenging assay[19]. DPPH, a commercially available oxidizing radical, is reduced by antioxidants. Purple colored DPPH was reduced to yellow colored diphenylpicrylhydrazyl. The disappearance of the DPPH radical absorption at a characteristic wavelength was monitored by decrease in optical density. For this purpose, different concentrations of Trolox solutions were prepared as standard. The sample extracts were mixed with same volume of 30% acetone and, from this diluted sample, varying volumes of extract (200, 400 and 800 μL) were taken to drum vials where 50% acetone was added to make the final volume up to 1 mL. Then 500 μL of 200 mmol/L 2-(N-morpholino)ethanesulfonic acid buffer was added to all vials followed by addition of 500 μL of 400 μmol/L DPPH solution (one by one at same interval). Simultaneously, sample blank was prepared for different concentrations of sample. Preparation of the sample blanks followed the same procedure described above, except ethanol was added instead of DPPH solution. After 20 min at ambient temperature, absorbance was measured at 520 nm in UV-spectrophotometer. A standard curve was constructed by plotting varying Trolox concentrations (0, 20, 40, 60 and 80 nmol/μL) on abscissa and absorbance on ordinates. The antioxidant capacity of the assayed samples was calculated by kinetics using following formula: $AC=m_2/m_1$, where m_1 is the slope of standard curve, and m_2 is the slope of triplicate sample. The results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC).

2.7. Statistical analysis

The assays were run in triplicate for each sample and the results expressed as mean value±SD. Pearson's correlation coefficient test was applied to test the association between the TPC and the AC of the samples analyzed using SPSS v16. Regression analysis was done using Microsoft Excel Spreadsheet to screen the plant samples with higher TPC and corresponding higher AC.

3. Results

3.1. TPC

The Table 2 represents the TPC in the hydrophilic extracts of 15 medicinal plants analyzed in this study. TPC is expressed as mg GAE/g FW. For the hydrophilic extracts of studied medicinal plants, variation in TPC contents was very large, up to 150 fold. TPC ranged from 0.04 mg to 6.01 mg GAE/g. *Z. rhetsa* had TPC (mean±SD) of (6.01±0.04) mg GAE/g that is the highest among all the study samples. The TPC content in the hydrophilic extracts of analyzed edible portions of the assayed plants was found in the order as *Z. rhetsa* > *O. corymbosa* > *S. tora* > *M. koenigi* > *A. sessilis* > *C. asiatica* > *O. corniculata* > *A. philoxeroides* > *M. oleifera* > *S. calva* > *A. viridis* > *P. retrofractum* > *P. angulata* > *C. benghalensis* > *A. spinosus*. Statistically, the TPC of these samples were significantly distributed in four groups using quartiles. Among 15 samples *Z. rhetsa*, *O. corymbosa* and *S. tora* were shown to have TPC above the 75th percentiles of all values.

Table 2
TPC and AC in selected medicinal plants.

English name	Scientific name	TPC (mg GAE/g FW)	TEAC (μmol TE/g FW)
Alligator weed	*A. philoxeroides*	0.56±0.03	0.14±0.00
Bengal dayflower	*C. benghalensis*	0.13±0.01	0.42±0.08
Creeping wood-sorrel	*O. corniculata*	0.61±0.02	3.47±1.17
Curry leaves	*M. koenigii*	1.18±0.08	1.13±0.14
Drumstick tree	*M. oleifera*	0.43±0.00	1.34±0.14
Gooseberry	*P. angulata*	0.20±0.00	0.41±0.03
Indian Ivy-rue	*Z. rhetsa*	6.01±0.04	7.54±0.00
Indian penny wort	*C. asiatica*	0.78±0.00	0.37±0.00
Javanese long pepper	*P. retrofractum*	0.22±0.01	1.24±0.21
Para cress	*S. calva*	0.43±0.03	0.19±0.03
Pinkwood sorrel	*O. corymbosa*	1.83±0.00	5.01±0.79
Sessile joyweed	*A. sessilis*	1.00±0.03	4.06±0.37
Sickle senna	*S. tora*	1.29±0.06	3.32±0.98
Slender amaranth	*A. viridis*	0.33±0.03	1.39±0.18
Spiny amaranth	*A. spinosus*	0.04±0.01	0.41±0.05

Values for TPC and TEAC are means±SD (n=3). TE: Trolox equivalent.

3.2. In vitro AC

The *in vitro* AC of the selected medicinal plants was determined from their hydrophilic extracts and the findings are presented in Table 2. Results for AC were expressed as μmol Trolox equivalent (TE)/g FW. As indicated in Table 2, all of the studied medicinal plants showed more or less AC. Unlike the TPC, there was much less variation, although up to 50 fold, in the measured AC of the studied plants. AC ranged from 0.14 μmol to 7.54 μmol TE/g of FW. The extract from leaves of *Z. rhetsa* displayed remarkably high levels of antioxidant potency. In fact, among all the study samples, *Z. rhesta* had the maximum AC (mean±SD) of (7.54±0.0) μmol TE/g of FW followed by (5.01±0.79) and (4.06±0.37) μmol TE/g of FW of *O. corymbosa* and *A. sessilis*, respectively. These three plants showed AC above the 75th percentiles of the capacities shown by all the studied plants. The AC shown by other 12 plants was found in the order as *O. corniculata* > *S. tora* > *A. viridis* > *M. oleifera* > *P. retrofractum* > *M. koenigii* > *C. benghalensis* > *A. spinosus* > *P. angulata* > *C. asiatica* > *S. calva* > *A. philoxeroides*.

3.3. Relationship between AC and TPC

The relationship between TPC and AC of the assayed hydrophilic extracts from the assayed plants was evaluated by regression analysis as shown in Figure 1. The results were significant: R^2=0.7, df=13, P<0.001. The equation of the regression line was AC (μmol TE/g FW)=0.784+1.2416×TPC (mg GAE/g FW). Interestingly, we found a R^2 value of 0.99 when the relationship between TPC and AC were modeled only for *Z. rhesta*, *A. sessilis* and *O. corymbosa*.

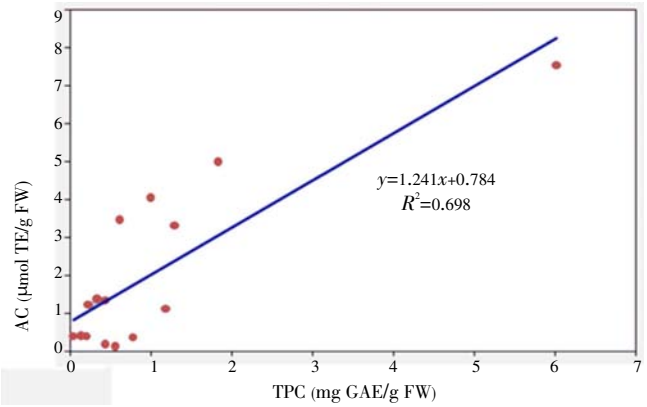

Figure 1. Correlation between TPC AC of the studied plants.

Apart from statistical correlation in Figure 1, a visual presentation of TPC and corresponding AC of each studied plants are provided in Figure 2.

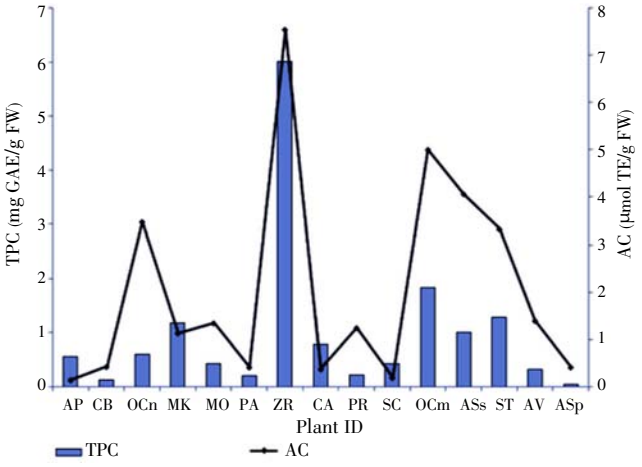

Figure 2. TPC and corresponding AC of assayed plants.
AP: *A. philoxeroides*; CB: *C. benghalensis*; OCn: *O. corniculata*; MK: *M. koenigii*; MO: *M. oleifera*; PA: *P. angulate*; ZR: *Z. rhetsa*; CA: *C. asiatica*; PR: *P. retrofractum*; SC: *S. calva*; OCm: *O. corniculata*; ASs: *A. sessilis*; ST: *S. tora*; AV: *A. viridis*; ASp: *A. spinosus*.

This figure revealed that AC of all the samples can't be solely explained by the TPC as shown in the cases of *O. corniculata*, *S. tora*, *A. viridis*, *A. sessilis*, *Z. rhetsa*, *M. oleifera*, *P. retrofractum*, *C. benghalensis*, *A. spinosus* and *P. angulata*. Figure 2 also explains that not all polyphenolic compounds have antioxidant potential as represented by the cases of *A. philoxeroides*, *C.. asiatica*, *S. calva* and *M. koenigii*.

4. Discussion

Free radicals are known to play an obvious role in several pathological manifestations. Antioxidants, either by counteracting these free radicals or protecting the body's antioxidant defense mechanisms, play a pivotal role in obtaining and preserving good health. Medicinal

plants, being the potential sources for a range of antioxidants in addition to their therapeutic properties, have attracted the attention of scientific researchers to meet the increasing demand for raw materials with natural potent antioxidants like polyphenols. Functionality of polyphenols is driven mainly by their special molecular structure consisting of several hydroxyl groups on aromatic rings. The mechanisms behind the antioxidant capacity of phenolic compounds are thought to involve breakdown of oxidative and nitrosative cascade and their capacity to function at cellular levels. These functions enable them to interact and modulate enzymatic activities and, thereby, regulate signal pathways for cell survival and death[12].

As the substances with comparatively higher antioxidant capacity *in vitro* are assumed to show higher antioxidant capacity *in vivo*, several *in vitro* techniques are commonly employed for rapid screening of the plant extracts based on their antioxidant capacity. In the present study, TPC was estimated by Folin-Ciocalteu assay as this method is simple, rapid and most popular. The Folin-Ciocalteu method is an electron transfer based assay, and gives reducing capacity which has normally been expressed as phenolic contents. Determination of antioxidant capacity was undertaken by employing the property of phenolic compounds and other natural antioxidants in the hydrophilic extracts of the studied plants to scavenge DPPH radical. The principle of this method lies in the reduction of DPPH and consequent loss of deep-purple color of DPPH solution in a response proportional to the concentration and potency of the plant extracts' antioxidant compounds that function as a proton radical scavenger or hydrogen donor[20].

In the present study, hydrophilic extracts of 15 medicinal plants contained varying amounts of TPC. This difference could be attributed to their physiological characteristics such as genetic variation, degree of maturity, color, and water content[21]. *Z. rhetsa*, *O. corymbosa* and *S. tora* contained the highest range (4th quartile) of TPC among the studied samples. To the authors' knowledge, no scientific reference sources are currently available to evaluate this comparatively higher TPC contents in these three plants. So, this study provides valuable preliminary data for screening polyphenols-rich medicinal plants as potential sources for future novel antioxidants. However, several groups of researchers have found more or less similar level of TPC in the medicinal plants of Nepal[22], Taiwan[23], India[24], Algeria[25], Jordan[26] and Peru[27]. In proportion to the results of TPC (R^2=0.99), *Z. rhesta*, *A. sessilis* and *O. corymbosa* showed the highest range (4th quartile) of antioxidant capacity among the studied plants. A fair correlation (R^2=0.7) between TPC and AC was observed when all of the 15 plants were modeled for correlating these two variables. Although the AC of the extracts from the studied medicinal plants has not been reported in any scientific reports before, the linear correlation between TPC and AC as observed in our study are well corroborated by several previous study findings[14-16,22,24,25,28,29]. So, the observed AC of the studied plants seems to be resulting, at least to a considerable extent if not entirely, from the redox properties of phenolic compounds. So, this study reveals that some medicinal plants, in addition to their current medicinal use, can serve as excellent sources of natural antioxidants.

To conclude, this study has shown that hydrophilic extracts of the studied medicinal plants had considerable level of antioxidant capacity with *Z. rhesta*, *A. sessilis* and *O. corymbosa* having the highest range of TPC and AC. Hence, the edible portions of these plants could be used as an excellent source of natural antioxidants in nutraceutical, food and medicinal industries. In addition, the inclusion of these plants to the regular diet for favoring antioxidant defense of the body is justified through this work. However, some phenolic compounds might have other roles rather than antioxidant function. Investigations into this area of isolating and identifying individual polyphenolic compounds and evaluating possible synergy among them for their antioxidant capacity by us are still in progress and seem likely to confirm our hypothesis that phenolic compounds significantly account for the antioxidant capacity of plant-derived foods.

Conflict of interest statement

We declare that we have no conflict of interest.

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